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  *                W E L C O M E   T O   T H E                  *
  *          U. S.   P A T E N T   T E X T   F I L E            *
  * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

=> s phosphoglycerate kinase(5A)promoter?

          1167 PHOSPHOGLYCERATE
          8093 KINASE
          1052 PHOSPHOGLYCERATE KINASE
                 (PHOSPHOGLYCERATE(W)KINASE)
         31965 PROMOTER?
L1         758 PHOSPHOGLYCERATE KINASE(5A)PROMOTER?

=> s l1 and adenovirus?

          3230 ADENOVIRUS?
L2         619 L1 AND ADENOVIRUS?

=> s l2 and tenacious

          2949 TENACIOUS
L3           1 L2 AND TENACIOUS

=> d l3,cit


1.  5,670,488, Sep. 23, 1997, Adenovirus vector for gene therapy;
Richard J. Gregory, et al., 514/44; 424/93.2; 435/320.1 [IMAGE AVAILABLE]

=> d kwic


US PAT NO:      5,670,488 [IMAGE AVAILABLE]            L3: 1 of 1
TITLE:          Adenovirus vector for gene therapy

SUMMARY:

BSUM(2)

 Cystic . . . responsible for 95% of the mortality. The first
manifestation of lung disease is often a cough, followed by progressive
dyspnea. Tenacious sputum becomes purulent because of colonization of
Staphylococcus and then with Pseudomonas. Chronic bronchitis and
bronchiectasis can be partially treated. . .

SUMMARY:

BSUM(13)

 The . . . in tissue culture, however, subsequent work has shown that
to deliver the gene to the airways of whole animals, defective
adenoviruses may be useful (Rosenfeld, (1992) Cell 68:143-155.
However, the safety and effectiveness of using defective adenoviruses
remain to be demonstrated.
```

SUMMARY:

BSUM(15)

In . . . transferring selected genetic material of interest (e.g., DNA or RNA) to cells in vivo. In preferred embodiments, the vectors are **adenovirus**-based. Advantages of **adenovirus**-based vectors for gene therapy are that they appear to be relatively safe and can be manipulated to encode the desired. . . the same time are inactivated in terms of their ability to replicate in a normal lytic viral life cycle. Additionally, **adenovirus** has a natural tropism for airway epithelia. Therefore, **adenovirus**-based vectors are particularly preferred for respiratory gene therapy applications such as gene therapy for cystic fibrosis.

SUMMARY:

BSUM(16)

In one embodiment, the **adenovirus**-based gene therapy vector comprises an **adenovirus** 2 serotype genome in which the Ela and Elb regions of the genome, which are involved in early stages of. . .

SUMMARY:

BSUM(17)

In another embodiment, the **adenovirus**-based therapy vector is a pseudo-**adenovirus** (PAV). PAVs contain no potentially harmful viral genes, have a theoretical capacity for foreign material of nearly 36 kb, may be produced in reasonably high titers and maintain the tropism of the parent **adenovirus** for dividing and non-dividing human target cell types. PAVs comprise **adenovirus** inverted terminal repeats and the minimal sequences of a wild-type **adenovirus** type 2 genome necessary for efficient replication and packaging by a helper virus and genetic material of interest. In a preferred embodiment, the PAV contains **adenovirus** 2 sequences.

SUMMARY:

BSUM(18)

In a further embodiment, the **adenovirus**-based gene therapy vector contains the open reading frame 6 (ORF6) of adenoviral early region 4 (E4) from the E4 promoter. . . all other E4 open reading frames. Optionally, this vector can include deletions in the E1 and/or E3 regions. Alteratively, the **adenovirus**-based gene therapy vector contains the open reading frame 3 (ORF3) of adenoviral E4 from the E4 promoter and is deleted. . . reducing the viability of the virus in cell culture. In combination with deletions in the E1 and/or E3 regions of **adenovirus** vectors, the theoretical insert capacity of the resultant vectors is increased to 8-9 kb.

DRAWING DESC:

DRWD(19)

FIG. 14 shows a map of the first generation **adenovirus** based vector encoding CFTR (Ad2/CFTR-1).

DRAWING DESC:

DRWD(21)

FIGS. 16A and 16B show a map of the second generation **adenovirus**

based vector, PAV.

DRAWING DESC:

DRWD(25)

FIGS. . . . in Rhesus monkeys after three vector administrations. These graphs demonstrate that all three monkeys treated with Ad2/CFTR-1 developed antibodies against **adenovirus**.

DRAWING DESC:

DRWD(27)

FIG. . . . a thickened basement membrane and occasional morphonuclear cells in the submucosa, but no abnormalities that could be attributed to the **adenovirus** vector.

DRAWING DESC:

DRWD(38)

FIGS. 33A-33C show antibody titers to **adenovirus** prior to and after the first and second administrations of Ad2-ORF6/PGK-CFTR. Prior to administration of Ad2-ORF6/PGK-CFTR, the monkeys had received. . .

DETDESC:

DETD(10)

In . . . issues arise. Many patients may have preexisting antibodies to some of the viruses that are candidates for vectors, for example, **adenoviruses**. In addition, repeated use of such vectors might induce an immune response. The use of defective viral vectors may alleviate. . .

DETDESC:

DETD(15)

Adeno-Associated Virus--(AAV) is a naturally occurring defective virus that requires other viruses such as **adenoviruses** or herpes viruses as helper viruses (Muzyczka, N. (1992) in Current Topics in Microbiology and Immunology 158:97). It is also. . .

DETDESC:

DETD(17)

Receptor . . . where much incoming material is degraded. One solution to this problem is the use of transferrin DNA-polylysine complexes linked to **adenovirus** capsids (Curiel, D. T. et al. (1991) Proc. Natl. Acad. Sci. USA 88:8850). The latter enter efficiently but have the. . . approach has promise but at present is relatively transient and suffers from the same potential problems of immunogenicity as other **adenovirus** based methods.

DETDESC:

DETD(18)

Adenovirus--Defective **adenoviruses** at present appear to be a promising approach to CF gene therapy (Berkner, K. L. (1988) BioTechniques 6:616). **Adenovirus** can be manipulated such that it encodes and expresses the desired gene product, (e.g., CFTR), and at the

same time is inactivated in terms of its ability to replicate in a normal lyric viral life cyc[redacted] In addition, **adenovirus** has a[redacted]tural tropism for airway epithelia. The viruses are able to infect quiescent cells as are found in the airways, offering a major advantage over retroviruses. **Adenovirus** expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, **adenoviruses** have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) Am. Rev. Respir. Dis. 109:233-238). Finally, **adenovirus** mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs. . . M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish **adenovirus** as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA. . .

DETDESC:

DETD(19)

The following properties would be desirable in the design of an **adenovirus** vector to transfer the gene for CFTR to the airway cells of a CF patient. The vector should allow sufficient. . . viral sequences and complementation to allow growth of the defective virus in the patient should be minimized. A first generation **adenovirus** vector encoding CFTR (Ad2/CFTR), made as described in the following Example 7, achieves most of these goals and was used. . .

DETDESC:

DETD(20)

FIG. . . . As can be seen from the figure, this first generation virus includes viral DNA derived from the common relatively benign **adenovirus** 2 serotype. The Ela and Elb regions of the viral genome, which are involved in early stages of viral replication. . .

DETDESC:

DETD(21)

The . . . Ela promoter. This is a moderately strong promoter that is functional in a variety of cells. In contrast to some **adenovirus** vectors (Rosenfeld, M. et al. (1992) Cell 68:143), this **adenovirus** retains the E3 viral coding region. As a consequence of the inclusion of E3, the length of the **adenovirus**-CFTR DNA is greater than that of the wild-type **adenovirus**. The greater length of the recombinant viral DNA renders it more difficult to package. This means that the growth of. . .

DETDESC:

DETD(24)

The **adenovirus** vector (Ad2/CFTR-1) and a related virus encoding the marker .beta.-galactosidase (Ad2/.beta.-gal) have been constructed and grown in human 293 cells. These cells contain the E1 region of **adenovirus** and constitutively express Ela and Elb, which complement the defective **adenoviruses** by providing the products of the genes deleted from the vector. Because the size of its genome is greater than. . .

DETDESC:

DETD(26)

Ad2/CFTR-1 is constructed from **adenovirus** 2 (Ad2) DNA sequences. Other varieties of **adenovirus** (e.g., Ad3, Ad5, and Ad7) may also prove useful as gene therapy vectors. This may prove essential if immune response. . .

DETDESC:

DETD(29)

Pseudo-**Adenovirus** Vectors (PAV)--PAVs contain **adenovirus** inverted terminal repeats and the minimal **adenovirus** 5' sequences required for helper virus dependent replication and packaging of the vector. These vectors contain no potentially harmful viral. . .

DETDESC:

DETD(30)

The . . . or as an infectious viral particle. As a plasmid construct, PAV is composed of the minimal sequences from wild type **adenovirus** type 2 necessary for efficient replication and packaging of these sequences and any desired additional exogenous genetic material, by either. . .

DETDESC:

DETD(31)

Specifically, PAV contains **adenovirus** 2 (Ad2) sequences as shown in FIG. 17, from nucleotide (nt) 0-356 forming the 5' end of the vector and. . .

DETDESC:

DETD(33)
Ad2-E4/ORF6 **Adenovirus** Vectors

DETDESC:

DETD(34)

An . . . lower levels of virus production. Therefore expressing ORF6, rather than ORF3, appears to be a better choice for producing recombinant **adenovirus** vectors.

DETDESC:

DETD(35)

The E4 region of **adenovirus** is suspected to have a role in viral DNA replication, late mRNA synthesis and host protein synthesis shut off, as well as in viral assembly (Falgout, B. and G. Ketner (1987) J. Virol. 61:3759-3768). **Adenovirus** early region 4 is required for efficient virus particle assembly. **Adenovirus** early region 4 encodes functions required for efficient DNA replication, late gene expression, and host cell shutoff. Halbert, D. N.. . .

DETDESC:

DETD(36)

The deletion of non-essential open reading frames of E4 increases the cloning capacity of recombinant **adenovirus** vectors by approximately 2

kb of insert DNA without significantly reducing the viability of the virus in cell cultur[redacted]When placed in combination wit[redacted]eletions in the E1 and/or E3 regions of **adenovirus** vectors, the theoretical insert capacity of the resultant vectors is increased to 8-9 kb. An example of where this increased. . . a result, this virus grows poorly and may occassionaly give rise to defective progeny. Including an E4 deletion in the **adenovirus** vector should alleviate these problems. In addition, it allows flexibility in the choice of promoters to drive CFTR expression from the virus. For example, strong promoters such as the **adenovirus** major late promoter, the cytomegalovirus immediate early promoter or a cellular promoter such as the CFTR promoter, which may be too large for first-generation **adenovirus** can be used to drive expression.

DETDESC:

DETD(80)

Generation of **Adenovirus** Vector Encoding CFTR (Ad2/CFTR)

DETDESC:

DETD(83)

The . . . the CFTR cDNA is shown as SEQ ID NO:2) was inserted between the Nhe1 and SnaB1 restriction sites of the **adenovirus** gene transfer vector pBR-Ad2-7. pBR-Ad2-7 is a pBR322 based plasmid containing an approximately 7 kb insert derived from the 5'. . .

DETDESC:

DETD(85)

To generate the recombinant Ad2/CFTR-1 **adenovirus**, the vector pBR-Ad2-7/CFTR was cleaved with BstB1 at the site corresponding to the unique BstB1 site at 10670 in Ad2.. . .

DETDESC:

DETD(88)

Ad2/CFTR-1 . . . human embryonal kidney cell line which were immortalized with sheared fragments of human Ad5 DNA. The 293 cell line expresses **adenovirus** early region 1 gene products and in consequence, will support the growth of E1 deficient **adenoviruses**. By analogy with retroviruses, 293 cells could be considered a packaging cell line, but they differ from usual retrovirus lines. . .

DETDESC:

DETD(97)

The . . . these correspond to a dose by mass of 0.25 .mu.g, 2.5 .mu.g and 6.25 .mu.g assuming a moleuclar mass for **adenovirus** of 150.times.10.sup.6.

DETDESC:

DETD(100)

Other . . . cells will be plated out in agar and examined for the appearance of transformed foci for 2 weeks. Wild type **adenovirus** will be used as a control.

DETDESC:

DETD(105)

Initial . . . involving the intratracheal instillation of the Ad-.beta.Gal viral vector into Syrian hamsters, which are reported to be permissive for human **adenovirus** are being performed. The first study, a time course assessment of the pulmonary and systemic acute inflammatory response to a. . .

DETDESC:

DETD(111)

Studies of recombinant **adenovirus** are also underway in primates. The goal of these studies is to assess the ability of recombinant adenoviral vectors to. . .

DETDESC:

DETD(113)

In . . . (.about.1 ml). This viral preparation was purified by CsCl gradient centrifugation and then by gel filtration chromatography one week later. **Adenoviruses** are typically stable in CsCl at 4.degree. C. for one to two weeks. However, this viral preparation was found to. . .

DETDESC:

DETD(114)

Monkeys . . . to establish baseline cell differentials and levels of .beta.-galactosidase. Blood was dram for baseline determination of cell differentials, blood chemistries, **adenovirus** antibody titers, and viral cultures. Each monkey was also examined for weight, temperature, appetite, and general health prior to infection.

DETDESC:

DETD(115)

The . . . B received the crude virus (.about.6 ml). (note that this was the second exposure of Monkey A to the recombinant **adenovirus**).

DETDESC:

DETD(119)

RT-PCR . . . indicates the presence of .beta.-galactosidase mRNA in cells obtained by brushings or swabs. PCR primers were used in both the **adenovirus** sequence and the LacZ sequence to distinguish virally-produced mRNA from endogenous mRNA. PCR was also used to detect the presence of the recombinant **adenovirus** DNA. Cytospin preparations was used to assess for the presence of virally produced .beta.-galactosidase mRNA in the respiratory epithelial cells. . .

DETDESC:

DETD(122)

Antibody titers to type 2 **adenovirus** and to the recombinant **adenovirus** were determined by ELISA. Blood/serum analysis was performed using an automated chemistry analyzer Hitachi 737 and an automated hematology analyzer Technicom H6. The blood buffy coat was cultured in A549 cells for wild type **adenovirus** and was cultured in the permissive 293 cells.

DETDESC:

DETD(134)
Adenovirus Vector

DETDESC:

DETD(135)

Ad2/CFTR-1 . . . transcripts. The recombinant virus E3 region was conserved. The size of the Ad2-CFTR-1 vector is approximately 104.5% that of wild-type **adenovirus**. The recombinant virus was grown in 293 cells that complement the E1 early viral promoters. The cells were frozen and. . .

DETDESC:

DETD(146)

Sera . . . obtained and anti-adenoviral antibody titers were measured by an enzyme-linked immunoadsorbant assay (ELISA). For the ELISA, 50 ng/well of filled **adenovirus** (Lee Biomolecular Research Laboratories, San Diego, Calif.) in 0.1M NaHCO.sub.3 were coated on 96 well plates at 4.degree. C. overnight.. . .

DETDESC:

DETD(147)

Neutralizing antibodies measure the ability of the monkey serum to prevent infection of 293 cells by **adenovirus**. Monkey serum (1:25 dilution) [or nasal washings (1:2 dilutions)] were added in two-fold serial dilutions to a 96 well plate. **Adenovirus** (2.5.times.10.sup.5 pfu was added and incubated for 1 hour at 37.degree. C. The 293 cells were then added to all. . .

DETDESC:

DETD(156)

Nested . . . sets were designed to selectively amplify Ad2/CFTR-1 DNA over endogenous CFTR by placing one primer from each set in the **adenovirus** sequence and the other primer in the CFTR sequence. The first primer set amplifies a 723 bp fragment and is. . .

DETDESC:

DETD(172)

To . . . reverse transcriptase was used to prepare cDNA from lung homogenates. The cDNA was amplified with PCR using primers that span **adenovirus** and CFTR-encoded sequences. Thus, the procedure did not detect endogenous rat CFTR. The lungs of animals which received Ad2/CFTR-1 were. . .

DETDESC:

DETD(176)

Because . . . is important to confirm this in vivo in the cotton rat, which is the most permissive animal model for human **adenovirus** infection (Ginsberg, H. S. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3823-3827; Prince, G. A. et al. (1993) J.. . .

DETDESC:

DETD(179)

It seemed possible that the recombinant **adenovirus** could escape from the lung into other tissues. To test for this possibility, other organs from the rats were evaluated. . .

DETDESC:

DETD(181)

Because **adenovirus** DNA integration into chromosomal DNA is not necessary for gene expression and only occurs at very low frequency, expression following any given treatments was anticipated to be finite and that repeated administration of recombinant **adenovirus** would be required for treatment of CF airway disease. Therefore, the effect of repeated administration of Ad2/CFTR-1 cotton rats was. . . after virus administration. At the time of the second vector administration all cotton rats had an increased antibody titer to **adenovirus**.

DETDESC:

DETD(182)

After . . . the rats died. Moreover, the results of studies assessing safety and efficacy were similar to results obtained in animals receiving **adenovirus** for the first time. Vital cultures of rat lung homogenates on 293 cells were negative at all time points, suggesting. . .

DETDESC:

DETD(187)

The . . . hybridize to the CFTR probe on Southern blot. This result shows the specificity of the reaction for amplification of the **adenovirus**-directed CFTR transcript.

DETDESC:

DETD(188)

The failure to detect evidence of **adenovirus**-encoded CFTR mRNA at 18 days or beyond suggests that the sensitivity of the RT-PCR may be low because of limited. . .

DETDESC:

DETD(192)

FIG. 20 shows that all three treated monkeys developed antibodies against **adenovirus**. Antibody titers measured by ELISA rose within two weeks after the first infection. With subsequent infections the titer rose within. . .

DETDESC:

DETD(196)

These results demonstrate the ability of a recombinant **adenovirus** encoding CFTR (Ad2/CFTR-1) to express CFTR cDNA in the airway epithelium of cotton rats and monkeys during repeated administration. They. . .

DETDESC:

DETD(198)

This study also provides the first comprehensive data on the safety of **adenovirus** vectors for gene transfer to airway epithelium. Several aspects of the studies are encouraging. There was no evidence of viral. . . replication of recombinant virus in humans will likely not be a problem. The other major consideration for safety of an **adenovirus** vector in the treatment of CF is the possibility of an inflammatory response. The data indicate that the virus generated. . . bronchoalveolar lavage and by brushing and swabs were not altered by virus application. Moreover, the histology of epithelia treated with **adenovirus** was indistinguishable from that of control epithelia. These data suggest that at least three sequential exposures of airway epithelium to **adenovirus** does not cause a detrimental inflammatory response.

DETDESC:

DETD(203)

Adenovirus vector. The recombinant **adenovirus** Ad2/CFTR-1 was used to deliver CFTR cDNA. The construction and preparation of Ad2/CFTR-1, and its use in vitro and in. . .

DETDESC:

DETD(205)

Three . . . (FEV1) greater than 50% of predicted and an arterial PO.sub.2 greater than 72. All patients were seropositive for type 2 **adenovirus**, and had no recent viral illnesses. Pretreatment cultures of nasal swabs, pharyngeal swabs, sputum, urine, stool, and blood leukocytes were negative for **adenovirus**. PCR of pretreatment nasal brushings using primers for the **adenovirus** E1 region were negative. Patients were evaluated at least twice by FEV1, cytology of nasal mucosa, visual inspection, and measurement. . .

DETDESC:

DETD(214)

On . . . sinus X-rays, pulmonary function tests, or arterial blood gases performed before and after Ad2/CFTR-1 administration. An increase in antibodies to **adenovirus** was not detectable by ELISA or by neutralization for 35 days after treatment.

DETDESC:

DETD(216)

Because . . . with CF--a thickened basement membrane and occasional polymorphonuclear cells in the submucosa--but no abnormalities that could be attributed to the **adenovirus** vector.

DETDESC:

DETD(223)
Efficacy of **Adenovirus**-Mediated Gene Transfer

DETDESC:

DETD(224)

The major conclusion of this study is that in vivo application of a recombinant **adenovirus** encoding CFTR can correct the defect in airway epithelial Cl.sup.- transport that is characteristic of CF epithelia.

DETDESC:

DETD(225)

Complementation . . . these parameters were detected for at least three weeks. These results represent the first report that administration of a recombinant **adenovirus** to humans can correct a genetic lesion as measured by a functional assay. This study contrasts with most earlier attempts. . .

DETDESC:

DETD(226)

Evidence . . . fluid secretion that was within the range observed in epithelia from normal subjects. At an MOI of 1, a related **adenovirus** vector produced .beta.-galactosidase activity in 20% of infected epithelial cells as assessed by fluorescence-activated cell analysis (Zabner et al. submitted for publication). Such data would imply that pharmacologic dose of **adenovirus** in CF airways might correspond to an MOI of one. If it is estimated that there are 2.times.10.sup.6 cells/cm.sup.2 in. . . 3.times.10.sup.9 potential target cells. Assuming a particle to I.U. ratio of 100, this would correspond to approximately 3.times.10.sup.11 particles of **adenovirus** with a mass of approximately 75 .mu.g. While obviously only a crude estimate, such information is useful in designing animal. . .

DETDESC:

DETD(227)

It is possible that an efficacious MOI of recombinant **adenovirus** could be less than the lowest MOI tested here. Some evidence suggests that not all cells in an epithelial monolayer. . .

DETDESC:

DETD(230)

Application of the **adenovirus** vector to the nasal epithelium in these three patients was well-tolerated. Although mild inflammation was observed in the nasal epithelium. . .

DETDESC:

DETD(231)

There . . . Nature Gen. (in press)). Replication only occurs in cells that supply the missing early proteins of the E1 region of **adenovirus**, such as 293 cells, or under conditions where the E1 region is provided by coinfection with or recombination with an E1-containing **adenovirus** (Graham, F. L. and Prevec, L. Vaccines: New Approaches to Immunological Problems (R. W. Ellis, ed., Boston, Butterworth-Heinermann, 1992); Berkner, K. L. (1988) Biotechniques 6:616-629). The patients studied here where seropositive for **adenovirus** types 2 and 5 prior to the study were negative for **adenovirus** upon culture of nasal swabs prior to administration of Ad2/CFTR-1, and were shown by PCR methods to lack endogenous E1. . .

DETDESC:

DETD(239)

A . . . contaminating helper virus. A novel protein IX, (pIX)

packaging system has been developed. This system exploits several documented features of **adenovirus** molecular biology. The first is that adenoviral defective particles are known to comprise up to 30% or more of standard. . .

DETDESC:

DETD(243)

Ad2-E4/ORF6 (FIG. 17 shows the plasmid construction of Ad2-E4/ORF6) is an **adenovirus** 2 based vector deleted for all Ad2 sequences between nucleotide 32815 and 35577. This deletion removes all open reading frames. . .

DETDESC:

DETD(244)

A . . . that E4 functions in the regulation of host cell protein synthesis and is therefore toxic to cells. Our current recombinant **adenoviruses** are deleted for the E1 region and must be grown in 293 cells which complement E1 functions. The E4 promoter. . .

DETDESC:

DETD(251)

This protocol uses a second generation **adenovirus** vector named Ad2-ORF6/PGK-CFTR. This virus lacks E1 and in its place contains a modified transcription unit with the **phosphoglycerate kinase** (PGK) **promoter** and a poly A addition site flanking the CFTR cDNA. The PGK promoter is of only moderate strength but is. . .

DETDESC:

DETD(252)

The . . . of the viral genome) have been deleted and replaced by an expression cassette encoding CFTR. The expression cassette includes the **promoter** for **phosphoglycerate kinase** (PGK) and a polyadenylation (poly A) addition signal from the bovine growth hormone gene (BGH). In addition, the E4 region. . . of Ad2 has been deleted and replaced with only open reading frame 6 (ORF6) of the Ad2 E4 region. The **Adenovirus** vector is referred to as AD2-ORF6/PGK-CFTR and is illustrated schematically in FIG. 28. The entire wild-type Ad2 genome has been previously sequenced (Roberts, R. J., (1986) In **Adenovirus** DNA, W. Oberfler, editor, Matinus Nihoff Publishing, Boston) and we have adopted the existing numbering system when referring to the. . .

DETDESC:

DETD(255)

At . . . the molecule will be confirmed. The remainder of the Ad2 viral DNA sequence is published in Roberts, R. J. in **Adenovirus** DNA. (W. Oberfler, Matinus Nihoff Publishing, Boston, 1986). The overall size of the Ad2-ORF6/PGK-CFTR vector is 36,336 bp which is. . .

DETDESC:

DETD(259)

In general terms, the vector is similar to several earlier **adenovirus** vectors encoding CFTR but it differs in three specific ways from our earlier Ad2/CFTR-1 construct.

DETDESC:

DETD(265)

A . . . deleted for two reasons. The first reason is to decrease the size of the vector used or expression of CFTR. **Adenovirus** vectors with genomes much larger than wild type are packaged less efficiently and are therefore difficult to grow to high. . .

DETDESC:

DETD(266)

The second reason to remove E4 sequences relates to the safety of **adenovirus** vectors. It is our goal to remove as many viral genes as possible to inactive the Ad2 virus backbone in. . . activation of the cellular transcription factor E2-F which is in turn implicated in the activation of the E2 region of **adenovirus** (Hemstrom et al. (1991) J. Virol., 65:1440-1449). Therefore removal of ORF6/7 from **adenovirus** vectors may provide a further margin of safety at least when grown in non-proliferating cells. The removal of the E1. . . ORF6 is believed to be involved in DNA replication, host cell shut off and late mRNA accumulation in the normal **adenovirus** life cycle. The E1-E4-ORF6.sup.+ backbone Ad2 vector does replicate in 293 cells.

DETDESC:

DETD(271)

Construction . . . Ad2-ORF6/PGK-CFTR virus was accomplished by in vivo recombination of Ad2-ORF6 DNA and a plasmid containing the 5' 10.7 Kb of **adenovirus** engineered to have an expression cassette encoding the human CFTR cDNA driven by the PGK promoter and a BGH poly. . .

DETDESC:

DETD(293)

Sera from the monkeys were obtained and antiadenoviral antibody titers were measured by ELISA. For the ELISA, 50 ng/well of killed **adenovirus** (Lee Biomolecular Research Laboratories, San Diego, Calif.) was coated in 0.1M NaHCO.sub.3 at 4.degree. C. overnight on 96 well plates.. . .

DETDESC:

DETD(312)

FIGS. 33A-33C show that all three monkeys had developed antibody titers to **adenovirus** prior to the first infection with Ad2-ORF6/PGK-CFTR (Zabner et al. (1993) Human Gene Therapy (in press)). Antibody titers measured by. . .

DETDESC:

DETD(313)

These results combined with demonstrate the ability of a recombinant **adenovirus** encoding CFTR (Ad2-ORF6/PGK-CFTR) to express CFTR cDNA in the airway epithelium of monkeys. These monkeys have been followed clinically for. . .

DETDESC:

DETD(314)

The . . . found no evidence of viral replication; infectious viral particles were rapidly cleared. The other major consideration for safety of an **adenovirus** vector in the treatment of CF is the possibility of an inflammatory response. The data indicate that the virus generated. . . been previously exposed three times to Ad2/CFTR-1, these data suggests that at least five sequential exposures of airway epithelium to **adenovirus** does not cause a detrimental inflammatory response.

CLAIMS:

CLMS(1)

We claim:

1. An adenoviral vector comprising an **adenovirus** genome from which one or more of the E4 open reading frames has been deleted, but retaining sufficient E4 sequences. . .

CLAIMS:

CLMS(3)

3. The vector of claim 1 from which the Ela and Elb regions of the **adenovirus** genome have been deleted.

CLAIMS:

CLMS(4)

4. The vector of claim 1 from which the E3 region of the **adenovirus** genome has been deleted.

CLAIMS:

CLMS(5)

5. The adenoviral vector of claim 1 in which open reading frame 6 of the E4 region is retained in the **adenovirus** genome.

CLAIMS:

CLMS(6)

6. The adenoviral vector of claim 1 in which open reading frame 3 of the E4 region is retained in the **adenovirus** genome.

CLAIMS:

CLMS(13)

13. . . . cystic fibrosis patient comprising administering directly to airway epithelial cells of the patient an adenoviral vector, said vector comprising an **adenovirus** genome from which one or more E4 open reading frames has been deleted, but retaining sufficient E4 sequences to promote. . .

CLAIMS:

CLMS(14)

14. The method of claim 13 wherein open reading frame 6 of the E4 region of the **adenovirus** genome is retained in the vector.

CLAIMS:

CLMS(16)

16. The method of claim 13 in which the Ela and Elb regions of the **adenovirus** genome of the vector have been deleted.

CLAIMS:

CLMS(17)

17. The method of claim 13 in which the E3 region of the **adenovirus** genome of the vector has been deleted.

CLAIMS:

CLMS(18)

18. The method of claim 13 wherein open reading frame 3 of the E4 region of the **adenovirus** genome is retained in the vector.